The supplementary material on the publication entitled "Isolation, characterisation and detection of breath-derived extracellular vesicles"

Garima Dobhal [1], Amrita Datta [2], Deanna Ayupova [2], Paul Teesdale-Spittle ,[3,4] and Renee V. Goreham [1*]

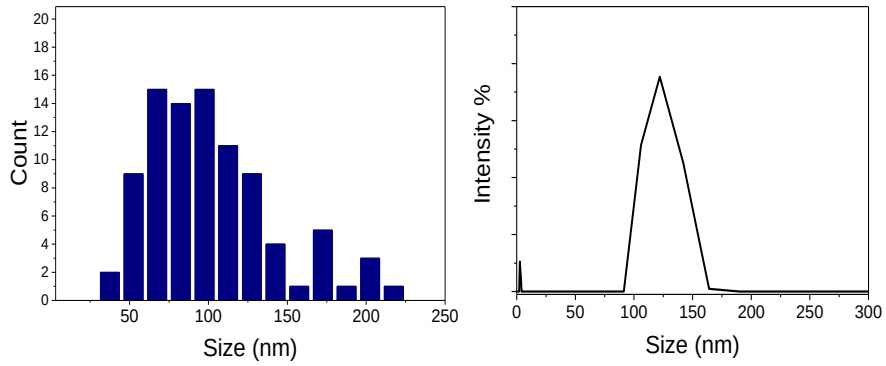
1 School of Mathematical and Physical Sciences, University of Newcastle, Callaghan 2308, Australia

2 School of Chemical and Physical Sciences, Victoria University of Wellington, Wellington 6012, New Zealand

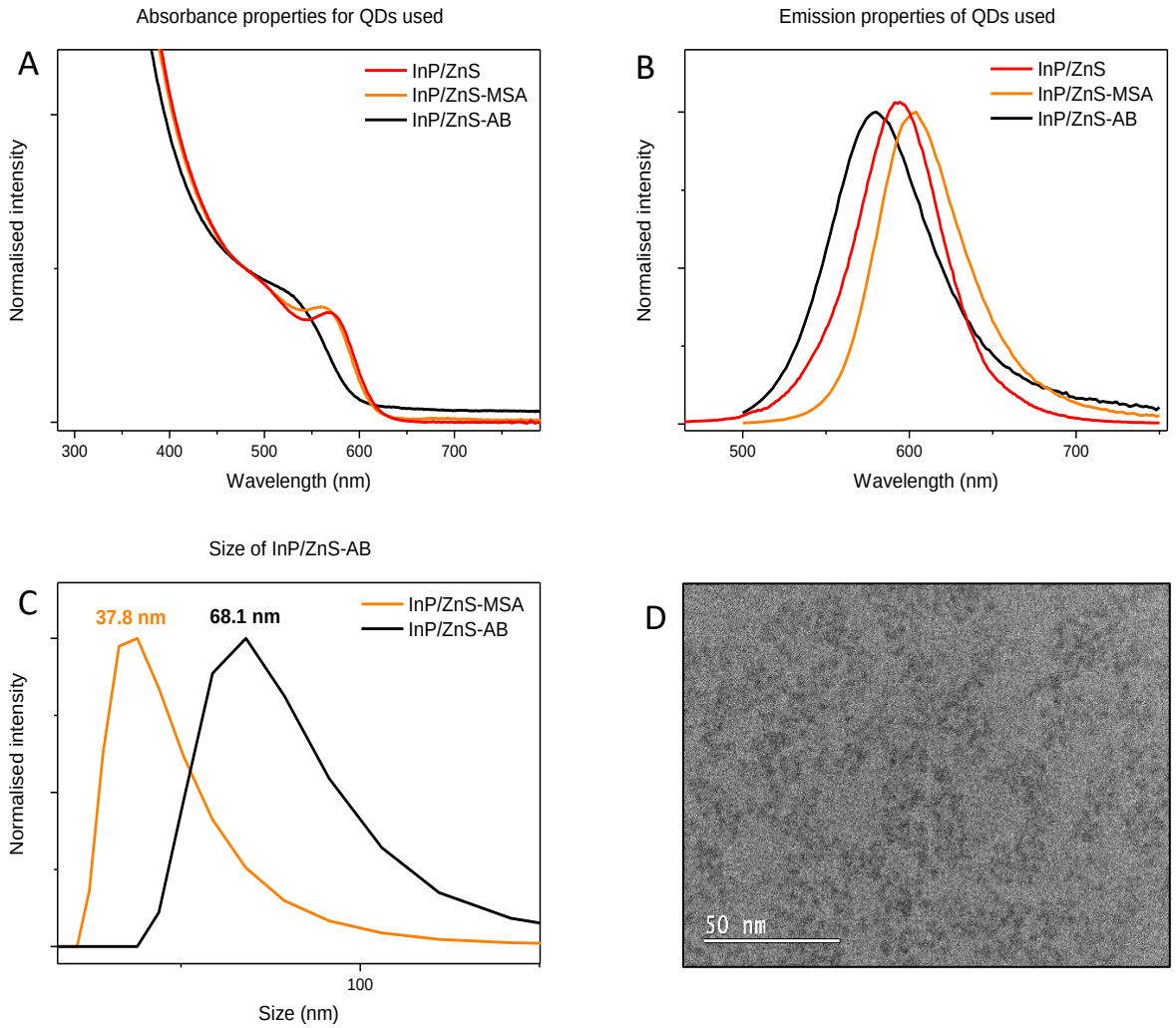
3 School of Biological Sciences, Victoria University of Wellington, Wellington 6012, New Zealand

4 Centre of Biodiscovery, Victoria University of Wellington, Wellington 6012, New Zealand

***** Correspondence: renee.goreham@newcastle.edu.au; Tel.: + 61 4 49138252



Supplementary figure S-1: (Left) Size distribution of 90 EBC derived EVs as measured using Cryo-SEM. (Right) Size by intensity measured using DLS.

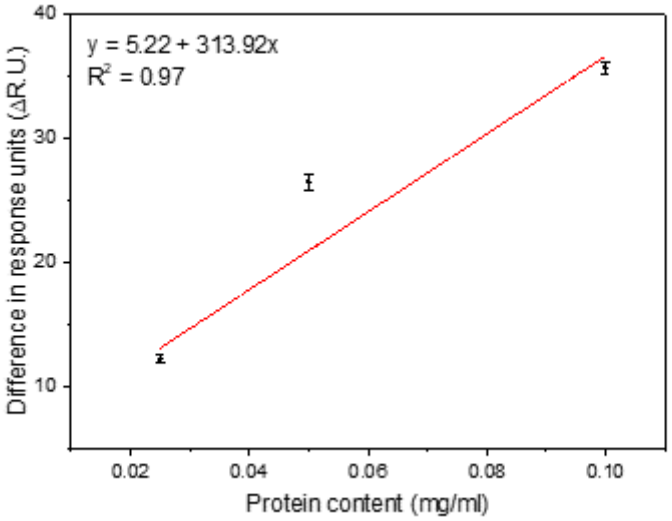


Supplementary figure S-2. (A) Absorbance profiles of the QDs used. (B) Emission profiles when excited at 480 nm. (C) Change in hydrodynamic size of the QDs post-conjugation as measured using DLS. (D) HR-TEM image of InP/ZnS QDs in toluene.

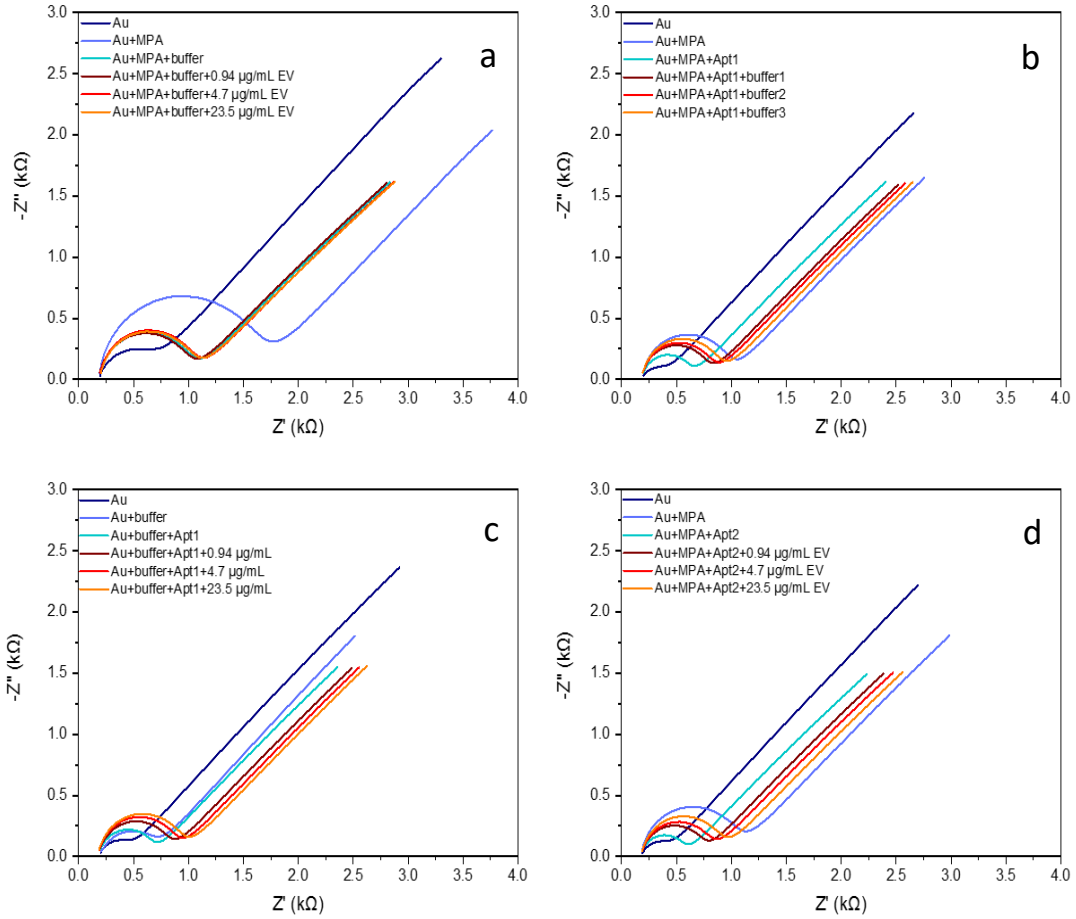
Supplementary table S-3:

Zeta potential and PLQY values for the InP/ZnS QDs used for SPR

Sample	Zeta Potential (mV)	PLQY (%)
InP/ZnS	-	25.45
InP/ZnS-MSA	-40.1	7.32
InP/ZnS-AB	-14.7	13.12



Supplementary figure S-4: Calibration curve for SPR of EBC EVs following a linear fit.



Supplementary figure S-5. Nyquist plots of impedance spectra representing the control experiments for when 10 mM PBS buffer replaces (a) Apt1; (b) three different concentrations of EVs; (c) MPA. (d) shows Apt1 being replaced by Apt2.

Supplementary table S-6:

Average charge transfer impedance values (R_{et}) and percentage errors associated with supplementary figure S-1 for the control experiments.

Aptamer control		
Layer	*R_{et}, Z' (Ω)*	*Error/ %*
Au	603.64	8.6035
Au+MPA	1480.10	1.6382
Au+MPA+buffer	884.35	2.7010
Au+MPA+buffer+0.26 µg/mL EV	865.51	2.7610
Au+MPA+buffer +1.3 µg/mL EV	916.23	2.5793
Au+MPA+buffer +6.5 µg/mL EV	906.52	2.9655
Concentrations of EV control		
Layer	*R_{et}, Z' (Ω)*	*Error/ %*
Au	309.05	2.9931
Au+MPA	825.45	1.7987
Au+MPA+Apt1	487.69	3.7671
Au+MPA+Apt1+buffer1	633.91	2.5297
Au+MPA+Apt1+buffer2	676.91	2.5360
Au+MPA+Apt1+buffer3	744.07	2.2623
MPA control		
Layer	*R_{et}, Z' (Ω)*	*Error/ %*
Au	469.12	5.2463
Au+buffer	561.53	2.4700
Au+buffer+Apt1	530.82	2.8088
Au+buffer+Apt1+0.26 µg/mL EV	673.69	2.2189
Au+buffer+Apt1+1.3 µg/mL EV	741.62	1.9306
Au+buffer+Apt1+6.5 µg/mL EV	797.70	1.8634
Specificity control		
Layer	*R_{et}, Z' (Ω)*	*Error/ %*
Au	269.95	5.2904
Au+MPA	899.66	2.1855
Au+MPA+Apt2	445.61	4.2470
Au+MPA+Apt2+0.26 µg/mL EV	599.20	2.8066
Au+MPA+Apt2+1.3 µg/mL EV	667.49	2.6989
Au+MPA+Apt2+6.5 µg/mL EV	761.71	2.2459

Supplementary methods:

Cell-derived exosome isolation

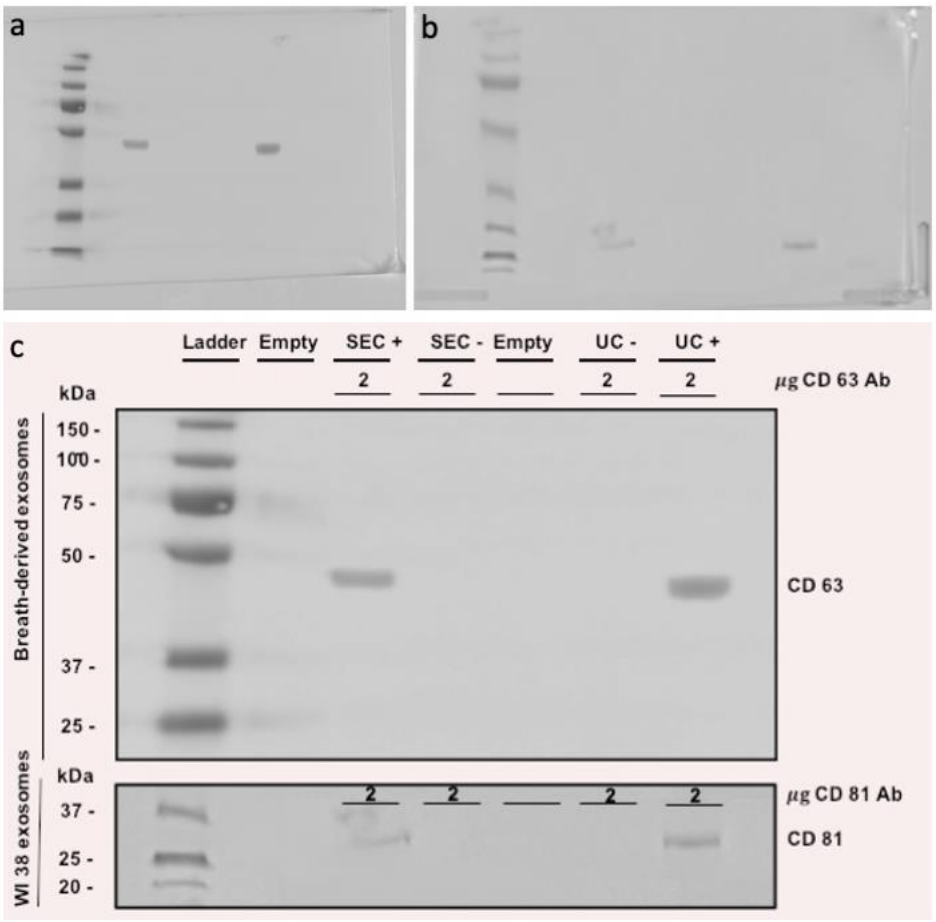
WI 38 cells were grown to 75% confluency under standard conditions, washed in PBS and incubated overnight in serum-free media. After 24 hours, vesicle-containing pellets were obtained from conditioned culture medium by ultracentrifugation as described by Lobb *et al*.[1] The pellets were resuspended in cold filtered (0.22 µm filter, Merck Millipore) PBS, pH 7.4, concentrated by centrifugal concentration (Amicon Ultra-4, Merck Millipore) and exosomes isolated by SEC Stranska *et al*.[2]

Exosomes purification and characterisation by Western Blot.

Samples from breath and conditioned cell culture media were assayed using western blotting to confirm the isolation procedure produced purified exosomes. Isolation of exosomes was performed as previously described, with blotting undertaken on samples from pooled fractions 7, 8, and 9 of SEC isolation using qEV original columns and from ultracentrifugation. Negative control samples free of exosomes were also assayed.

Samples were suspended in lysis buffer (50mM Tris-HCl pH 8.0, 150mM NaCl, 1% NP-40) and subjected to two rounds of vortex vigorously (10 s) and cooling on ice (20-30 min). Samples were finally centrifuged at 15,000 x*g* for 10 min. The pellet was discarded and the protein concentration of the supernatant was determined using a micro-BCA assay (Pierce BCA Protein Assay Kit) according to the manufacturer's instructions. Samples containing approximately 20 µg of protein were loaded on a 10% acrylamide gel, and proteins separated by electrophoresis.

The western blot was performed according to the protocol described by Lim *et al.* [3] with minor amendments: 5% bovine serum albumin was used as a blocking agent; the membrane was probed with anti-CD63 at 1:250 dilution (TS63; Invitrogen, ref. 10628D), anti-CD81 at 1:250 dilution (M38; Invitrogen, ref.10630D) and secondary mouse monoclonal secondary antibody conjugated to horseradish peroxidase at 1:5000 dilution (Santa Cruz, sc-2005). The membrane was visualized on an Amersham Imager 600 (General Electric).



Supplementary figure S-7. Western blot analysis using common exosome markers CD63 and CD81 in exosomes derived from breath and WI38 cells. Samples that contain exosomes from size exclusion chromatography or ultracentrifugation are labelled SEC+ and UC+, respectively. (a) shows the raw gel image for CD63 marker and (b) shows the raw gel image for CD81 marker. (c) Exosome-free negative controls are also shown. These are samples of matching total protein content to the exosome samples but are from exosome-free size exclusion or ultracentrifugation fractions, and are labelled SEC- and UC-. The markers demonstrate that the methodology isolates exosomes from breath equivalently to the established isolation from cell culture conditioned media. Total loaded protein lysate ~ 20 µg/lane for all samples including negative controls. Blotting was performed using 2 µg/mL of anti-CD63 and anti-CD81. The goat anti-mouse secondary antibody was used at a concentration of 0.08 µg/mL.

References

1. Lobb RJ, Becker M, Wen SW, et al (2015) Optimized exosome isolation protocol for cell culture supernatant and human plasma. J Extracell Vesicles 4:27031. https://doi.org/10.3402/jev.v4.27031

2. Stranska R, Gysbrechts L, Wouters J, et al (2018) Comparison of membrane affinity-based method with size-exclusion chromatography for isolation of

exosome-like vesicles from human plasma. J Transl Med 16:1. https://doi.org/10.1186/s12967-017-1374-6

3. Lim J, Choi M, Lee H, et al (2019) Direct isolation and characterization of circulating exosomes from biological samples using magnetic nanowires. J Nanobiotechnology 17:1. https://doi.org/10.1186/s12951-018-0433-3